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16. ABSTRACT

Ethylene thiourea (ETU) was fed to groups of rats at 0, 1, 5, 125 or 625 ppm for up to 90 days. Other groups of rats received either propylthiouracil (PTU; 125 ppm) or amitrole (50 ppm) in their diets as positive controls. Only those rats which received ETU at 125 or 625 ppm and those ingesting PTU or amitrole demonstrated a measurable toxic response. This toxicity was reflected as an alteration in thyroid function and a significant change in thyroid morphology.

Ingestion of 625 ppm ETU or 125 ppm PTU resulted in very substantial decreases in serum triiodothyronine (T-3) and thyroxine (T-4). Marked increases in serum thyroid stimulating hormone (TSH) levels were found in the 625 and 125 ppm ETU rats, the 125 PTU rats, and the rats receiving amitrole, each time this hormone was measured. Rats which ingested 625 ppm ETU also exhibited a decrease in iodide uptake by the thyroid. While a statistically significant increase in serum T-4 and degree of thyroid hyperplasia was observed for rats ingesting 25 ppm ETU for 60 days, normal thyroid hormone levels and thyroid morphology was found in rats on 25 ppm ETU for either 30 or 90 days.

Based on biochemical and microscopic changes examined, the no-effect level for dietary ETU in this 90-day study is considered to be 25 ppm.

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DIETARY SUBACUTE TOXICITY OF ETHYLENE THIOUREA IN THE LABORATORY RAT

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Ingestion of 625 ppm ETU or 125 ppm PTU resulted in very substantial decreases in serum triiodothyronine (T-3) and thyroxine (T-4). Marked increases in serum thyroid stimulating hormone (TSH) levels were found in the 625 and 125 ppm ETU rats, the 125 PTU rats, and the rats receiving amitrole, each time this hormone was measured. Rats which ingested 625 ppm ETU also exhibited a decrease in iodide uptake by the thyroid. While a statistically significant increase in serum T-4 and degree of thyroid hyperplasia was observed for rats ingesting 25 ppm ETU for 60 days, normal thyroid hormone levels and thyroid morphology was found in rats on 25 ppm ETU for either 30 or 90 days.

Based on biochemical and microscopic changes examined, the no-effect level for dietary ETU in this 90-day study is considered to be 25 ppm.

INTRODUCTION

The salts of ethylenebisdithiocarbamic acid (EBDC) have been extensively used against a variety of pathogenic plant fungi. A principal impurity and degradation product of the EBDC fungicides is ethylene thiourea (2-imidazolidinethione; ETU). ETU has been identified as an impurity in commercial EBDC formulations (Clarke *et al.*, 1951; Bontoyan *et al.*, 1972). It has been reported to occur as a result of metabolic (Engst and Schnaak,

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1967; Seidler *et al.*, 1970; Truhaut *et al.*, 1973) and chemical (Lopatecki and Newton, 1952; Fishbein and Fawkes, 1965; Engst and Schnaak, 1974) alterations of the commercial fungicides. ETU has been identified on a number of different crops which had been field-sprayed with a commercial formulation of EBDC (Yip *et al.*, 1971; Newsome, 1972). Cooking of foods containing EBDC residues also results in the formulation of ETU (Newsome and Laver, 1973; Watts *et al.*, 1974).

Early studies evaluating the toxicity of the EBDC fungicides reported a major effect to be thyroid hyperplasia and neoplasia (Blackwell – Smith *et al.*, 1953; Andrianova and Alekseev, 1970). The toxicity of ETU has also been studied and found to be essentially similar to that of the parent fungicide, producing thyroid neoplasia in rats (Ulland *et al.*, 1972; Graham *et al.*, 1975) and liver tumors in mice (Innes *et al.*, 1969). In these published reports, recorded parameters consisted of body weights, organ weights, food consumption, ^{131}I uptake, and terminal histopathology. In the ETU subacute toxicity study reported herein, thyroid hormone level measurements were also made to determine at what dietary dose level ETU causes biochemical (thyroid hormone) alterations and to establish a dietary concentration which does not induce thyroid changes leading to hyperplasia. In addition, it was hoped that a relationship between altered thyroid hormone levels and thyroid hyperplasia could be established.

MATERIALS AND METHODS

Sprague – Dawley-derived rats were purchased from Charles River Laboratories (Wilmington, MA); ETU was obtained from the Aldrich Chemical Company (Milwaukee, WI). The ETU used in this study was analyzed by mass spectroscopy and nuclear magnetic resonance spectrometry (NMR). Direct probe high resolution mass spectroscopy showed a strong parent ion at m/e 103. It was estimated through the use of both ^{13}C and ^{2}H NMR spectroscopy that ETU was 96.8 percent pure. Propylthiouracil (PTU) and amitrole (3-amino-1, 2, 4-triazole) were purchased from the Sigma Chemical Company (St. Louis, MO). ^{125}I for the thyroid iodine uptake studies was obtained from New England Nuclear (Boston, MA). Test kits for measuring serum thyroxine (T-4) and thyroid binding globulin (TBG) were purchased from Nuclear Medical Laboratories, Inc. (Dallas, TX). Triiodothyronine (T-3) kits were obtaind from Diagnostics Products Corporation (Los Angeles, CA). Initial serum TSH measurements were performed with kits purchased from Beckman Instruments, Inc. (Fullerton, CA). A TSH kit from Calbiochem (San Diego, CA) was used at the end of this study.

ETU, PTU, or amitrole was added to corn oil and then incorporated into rodent feed (Ralston Purina Company, Richmond, Indiana) using a twin shell tumble blender. The final corn oil concentration in the blended feed was 1 percent. The test diets were prepared fresh weekly. The dietary levels of ETU were 625, 125, 25, 5 and 1 ppm; PTU was 125 ppm, and amitrole, 50 ppm. These dietary levels of PTU and amitrole were previously shown to produce significant antithyroid effects (Griessen and Lemarchand – Beraud, 1973; Seifter and Ehrich, 1948). A control group received powdered

diet containing 1 percent corn oil. Each of the seven dosage groups contained 60 male and 60 female rats. The control group contained 72 male and 72 female rats. The animals were placed on study as outlined in Table 1.

TABLE 1. ETU Protocol Design

ETU (ppm)	Sex	Number of Rats/Treatment Period		
		30 Days	60 Days	90 Days
625	M	20	20	20
625	F	20	20	20
125	M	20	20	20
125	F	20	20	20
25	M	20	20	20
25	F	20	20	20
5	M	20	20	20
5	F	20	20	20
1	M	20	20	20
1	F	20	20	20
0	M	24	24	24
0	F	24	24	24
Amitrole (ppm)				
50	M	20	20	20
50	F	20	20	20
PTU (ppm)				
125	M	20	20	20
125	F	20	20	20

At 30-day intervals, 10 rats of each sex from each test group were sacrificed and serum T-3, T-4, TBG, and TSH concentrations were measured. These rats were also used for necropsy. The remaining 10 rats of each sex per group were used for thyroid ^{125}I uptake studies. Each rat was individually caged throughout the study. Body weight and food consumption were determined weekly.

The tissues removed during necropsy were heart, spleen, kidney, ovary (paired), testicle (paired), liver, pituitary, thyroid (paired), adrenal (paired), and brain. Due to excessive salivation and abnormal skin-hair coat development at the 625 ppm ETU level, skin and mandibular salivary glands were also removed during necropsy from the rats which received the high diet. The thyroid gland and liver were examined microscopically in all study groups. These two tissues were fixed in formalin imbedded in paraffin, sectioned (6 microns), and stained with Hematoxylin-Eosin. The remaining tissues were stored in 10 percent buffered formalin.

RESULTS AND DISCUSSION

Mortality was observed in this study only at the highest ETU dose level. Fourteen of the 40 rats on the 625 ppm ETU 60-day study died between days 40 and 60 of the study. Only one rat from the 625 ppm 90-day group died.

There was no mortality noted in this study attributable to the presence of ETU in the diet at dietary levels of 125 ppm and below. Growth, as evidenced by weekly recording of body weight data, was normal at dietary levels of 125 ppm and below. Only those rats which received 625 ppm ETU or 125 ppm PTU test diets showed a marked decrease in body weight gain. No difference in body weight gain was observed for those rats which received ETU at 125, 25, 5 or 1 ppm, or amitrole, as compared to the control group (Fig. 1).

The rats which received 625 ppm ETU showed clinical signs of poisoning by the eighth day on study. Excessive salivation, loss of hair or failure to replace normal hair loss, rough and bristly hair coat, and scaly skin texture were observed. Ingestion of ETU was calculated and found to range from 11.7 to 15.2 mg/day in female rats to 13.0 to 17.3 mg/day in male rats. Table 2 shows data on the daily consumption of ETU and other chemicals by all animals on study expressed as mg/kg body weight.

The rats receiving 125, 25, 5, 1 and 0 ppm ETU, 50 ppm amitrole, or 125 ppm PTU showed no adverse clinical effects.

Altered thyroid function can result from the action of a chemical on iodine uptake and incorporation, synthesis or release of thyroid hormones, synthesis or release of TSH, or inactivation of receptors activated by either TSH (at the level of the thyroid) or by thyroid hormone (at the pituitary level). Using commercially available test kits, the serum concentration of T-3, T-4, TBG, and TSH can be measured. Unfortunately, after the ETU subacture toxicity study began, the manufacturer of the kit used to measure TSH switched to a new antiserum with increased specificity for human TSH. This kit would no longer measure rat TSH. Therefore, TSH kits from other commercial sources were purchased and evaluated as possible alternates. The kit produced by Calbiochem contained a relatively nonspecific TSH antiserum and was used to measure rat serum TSH levels. Unfortunately, this was not confirmed until near the end of the study and for this reason, TSH values are available for only very few groups.

^{125}I uptake can be measured directly. The Free Thyroxine Index (FTI) was calculated and is reported for each group. The FTI is a measurement of free serum T-4. While the T-4 level, measured by the T-4 radioimmunoassay kit, is influenced by TBG concentrations, the FTI is a measurement of the amount of T-4 free in the serum, independent of the serum TBG concentration. The formula used to calculate the FTI is shown below:

$$\text{FTI} = \frac{\text{(T-3\% Bound)}}{100} \times \text{T-4} \quad \text{g\%}$$

Consistent with the observable clinical changes, several biochemical changes were noted. Tables 3, 4, and 5 list the percent iodine uptake and the TBG, T-3, and T-4 concentrations after 30, 60, and 90 days on study, respectively. Also included in these tables are the values for the amitrole and PTU positive controls. TSH values are given for those test groups in which it was measured. The calculated FTI for each test group is shown in Table 6.

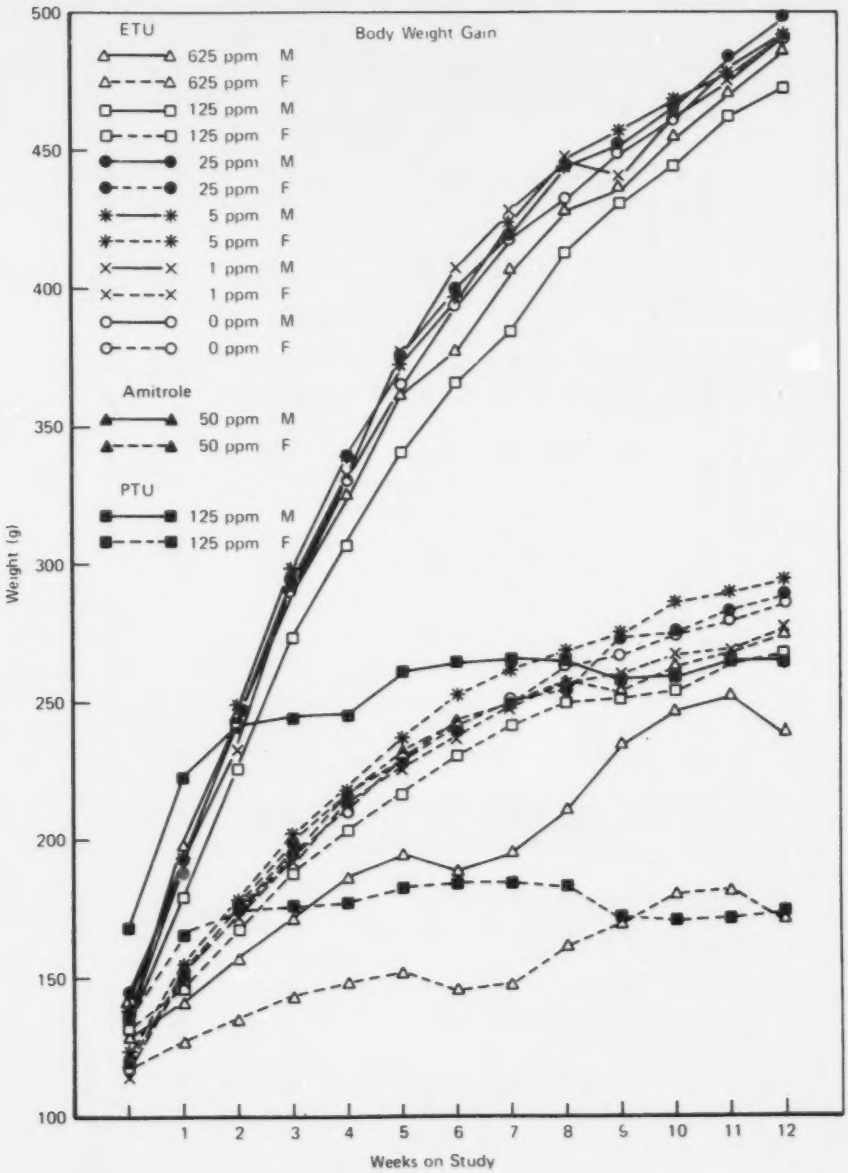

FIGURE 1. Body Weight Gain

TABLE 2. ETU Ingestion[1]

ETU (PPM)	Sex	Week No. 1	Week No. 2	Week No. 3	Week No. 4	Week No. 5	Week No. 6	Week No. 7	Week No. 8	Week No. 9	Week No. 10	Week No. 11	Week No. 12
625	M	57.57	64.85	52.87	47.99	45.14	47.50	49.20	50.01	44.79	43.85	40.77	34.22
625	F	59.63	68.42	52.10	50.48	48.10	52.23	55.58	58.01	55.60	50.25	46.21	46.72
125	M	11.89	11.15	9.96	8.77	8.96	7.76	10.33	7.71	6.97	7.10	7.01	6.21
125	F	12.90	11.48	10.96	11.13	11.24	9.67	8.44	8.63	11.63	10.27	9.19	8.63
25	M	2.19	2.24	1.99	1.79	1.59	1.53	1.60	1.49	1.55	1.35	1.48	1.44
25	F	2.26	2.43	2.36	2.10	1.81	1.78	2.00	1.97	1.88	1.71	1.92	2.12
5	M	0.51	0.46	0.38	0.38	0.35	0.32	0.30	0.29	0.28	0.24	0.27	0.29
5	F	0.56	0.48	0.43	0.46	0.36	0.34	0.33	0.37	0.36	0.32	0.40	0.29
1	M	0.07	0.06	0.10	0.09	0.08	0.07	0.07	0.07	0.07	0.06	0.06	0.06
1	F	0.09	0.08	0.07	0.07	0.06	0.06	0.06	0.06	0.11	0.11	0.11	0.05
0	M	0	0	0	0	0	0	0	0	0	0	0	0
0	F	0	0	0	0	0	0	0	0	0	0	0	0
Amitrole (PPM)													
50	M	4.94	4.26	3.97	3.61	3.48	3.21	3.13	3.03	3.76	3.60	2.51	2.81
50	F	5.23	4.77	4.69	4.42	4.00	3.76	3.94	3.70	3.64	4.71	3.13	3.58
PTU (PPM)													
125	M	11.98	10.09	8.88	7.22	7.09	6.52	6.90	6.50	7.00	7.00	6.14	6.19
125	F	12.86	11.15	9.64	7.47	8.41	8.58	9.20	8.86	10.06	9.28	7.13	9.00

[1] Mean data expressed as mg/kg body weight/day

TABLE 3. Thyroid Hormone Levels — After 30 Days On Study

ETU (ppm)	Sex	^{125}I (percent uptake)	TBG (percent T-3 bound)	T-3 (ng percent)	T-4 (µg percent)	TSH (µIU per ml)
625	M	1.2 ± 0.4[1]	60.3 ± 2.6[1]	57.3 ± 3.7[1]	0.9 ± 0.6[1]	14.3 ± 0.9[1]
625	F	2.1 ± 1.4[1]	60.6 ± 1.8[1]	58.4 ± 9.9[1]	1.1 ± 1.0[1]	14.6 ± 1.9[1]
125	M	3.6 ± 0.8	62.7 ± 1.3[1]	71.1 ± 11.8	2.6 ± 0.4[1]	23.3 ± 5.9[1]
125	F	4.0 ± 1.7	61.5 ± 1.3[1]	104.4 ± 16.3[1]	2.1 ± 0.5[1]	18.3 ± 4.0[1]
25	M	2.9 ± 0.6	65.7 ± 2.3	67.1 ± 15.9	5.6 ± 1.1	7.3 ± 1.5
25	F	3.2 ± 1.3	63.5 ± 2.0	86.3 ± 14.8	3.8 ± 0.8	5.1 ± 1.3
5	M	3.6 ± 0.6	69.3 ± 6.3	79.0 ± 8.1	4.7 ± 0.4	6.7 ± 1.4
5	F	3.8 ± 1.0	68.9 ± 1.3	88.1 ± 12.8	2.9 ± 0.9	4.9 ± 1.4
1	M	3.7 ± 0.7	64.5 ± 1.2	82.1 ± 13.0	5.1 ± 1.0	6.4 ± 0.8
1	F	3.0 ± 0.5	63.4 ± 1.3	90.9 ± 11.3	3.5 ± 1.0	4.5 ± 0.9
0	M	3.6 ± 0.9	68.0 ± 5.6	76.0 ± 11.8	5.0 ± 1.7	6.7 ± 2.5
0	F	3.5 ± 0.9	66.0 ± 5.2	83.2 ± 16.2	3.8 ± 1.4	6.0 ± 4.1
Amitrole (ppm)						
50	M	3.7 ± 2.1	63.0 ± 1.5	49.1 ± 19.5[1]	0.6 ± 0.8[1]	9.3 ± 0.6[1]
50	F	5.0 ± 3.5	61.4 ± 1.0[1]	55.2 ± 7.2[1]	0.3 ± 0.3[1]	8.9 ± 0.6[1]
PTU (ppm)						
125	M	2.9 ± 1.1	67.8 ± 2.0	58.9 ± 6.1[1]	0.9 ± 0.2[1]	- -
125	F	3.3 ± 0.7	69.5 ± 1.6	52.0 ± 8.0[1]	0.7 ± 0.1[1]	

[1] Significantly different ($p < 0.05$) from corresponding control. Student's test was used to make comparison between the control and treated animals. All data reported as the mean, ± S.D.

TABLE 4. Thyroid Hormone Levels — After 60 Days On Study

ETU (ppm)	Sex	^{125}I (percent uptake)	TBG (percent T-3 bound)	T-3 (ng Percent)	T-4 (µg percent)	TSH (µIU per ml)
625	M	1.9 ± 1.0[1]	79.0 ± 0.9	56.9 ± 10.3[1]	0.2 ± 0.1[1]	-
625	F	2.4 ± 1.8[1]	71.8 ± 1.4	56.8 ± 6.9[1]	0.2 ± 0.1[1]	-
125	M	3.6 ± 1.4	66.3 ± 1.3	79.8 ± 28.1	2.8 ± 0.5[1]	
125	F	3.3 ± 1.0	66.3 ± 2.1	78.5 ± 28.6[1]	2.0 ± 0.5[1]	-
25	M	3.2 ± 0.7	76.9 ± 1.6	86.4 ± 7.6	2.8 ± 0.5[1]	-
25	F	3.7 ± 1.3	74.7 ± 1.7	126.2 ± 15.1	2.6 ± 0.5[1]	-
5	M	3.5 ± 0.8	66.4 ± 1.2	85.4 ± 12.7	4.9 ± 0.5	-
5	F	4.0 ± 0.8	64.0 ± 1.8	118.5 ± 14.3	2.9 ± 0.9	-
1	M	2.7 ± 0.6	70.4 ± 1.2	80.3 ± 12.0	4.9 ± 0.7	-
1	F	3.2 ± 0.7	67.1 ± 1.3	93.3 ± 13.5	2.8 ± 0.8	-
0	M	4.3 ± 0.9	73.6 ± 4.9	77.3 ± 8.5	4.8 ± 0.7	5.8 ± 0.4[2]
0	F	3.5 ± 0.8	69.4 ± 4.3	103.8 ± 19.1	3.3 ± 0.5	6.4 ± 0.9[2]
Amitrole (ppm)						
50	M	4.0 ± 0.7	74.8 ± 1.6	83.4 ± 8.9	5.9 ± 0.8	-
50	F	4.8 ± 0.9	69.8 ± 1.3	111.6 ± 10.7	3.5 ± 0.6	-
PTU (ppm)						
125	M	3.9 ± 1.6	61.7 ± 2.6[1]	46.1 ± 3.9[1]	1.2 ± 0.2[1]	9.8 ± 1.0[1]
125	F	5.4 ± 1.7	62.2 ± 2.1[1]	50.9 ± 9.7[1]	0.8 ± 6.1[1]	10.8 ± 1.9[1]

[1] Significantly different ($p < 0.05$) from correcponding control.

[2] TSH values to be used as control for PTU group.

TABLE 5. Thyroid Hormone Levels — After 90 Days On Study

ETU (ppm)	Sex	^{125}I (percent uptake)	TBG (percent T-3 bound)	T-3 (ng percent)	T-4 (µg percent)	TSH (µIU per ml)
625	M	2.5 ± 0.8[1]	62.7 ± 2.0[1]	27.9 ± 13.3[1]	1.1 ± 0.6[1]	-
625	F	3.7 ± 1.8	62.7 ± 0.9[1]	35.2 ± 4.3[1]	1.1 ± 0.6[1]	-
125	M	2.8 ± 0.7	65.3 ± 1.1	86.1 ± 15.0	2.3 ± 0.6[1]	-
125	F	3.9 ± 1.1	64.3 ± 1.6	105.5 ± 16.0	1.6 ± 0.3[1]	-
25	M	3.3 ± 0.7	68.9 ± 1.5	79.4 ± 12.6	3.8 ± 1.0	-
25	F	3.4 ± 0.9	65.6 ± 2.3	108.7 ± 11.6	2.9 ± 0.7	-
5	M	3.7 ± 0.6	71.4 ± 0.8	76.1 ± 13.1	5.0 ± 1.0	-
5	F	4.2 ± 1.1	70.1 ± 2.2	105.2 ± 16.6	3.0 ± 0.7	-
1	M	3.5 ± 0.6	65.8 ± 1.1	68.7 ± 9.9	4.0 ± 1.0	-
1	F	3.2 ± 0.9	63.1 ± 1.4	116.7 ± 17.6	2.5 ± 0.7	-
0	M	3.8 ± 0.5	69.3 ± 2.7	72.0 ± 21.5	4.5 ± 0.8	5.8 ± 0.4[2]
0	F	4.1 ± 1.0	65.2 ± 2.9	106.8 ± 25.0	3.3 ± 0.8	6.4 ± 0.9[2]
Amitrole (ppm)						
50	M	5.8 ± 3.1	71.4 ± 1.5	67.3 ± 11.8	3.6 ± 1.3	-
50	F	4.8 ± 3.2	69.6 ± 2.1	94.8 ± 13.3	3.5 ± 1.1	-
PTU (ppm)						
125	M	4.7 ± 1.7	59.1 ± 1.3[1]	73.2 ± 9.9	0.6 ± 0.2[1]	9.4 ± 1.3[1]
125	F	5.6 ± 2.5	60.4 ± 1.7[1]	69.6 ± 9.4[1]	0.4 ± 0.2[1]	10.7 ± 2.1[1]

[1] Significantly different ($p < 0.05$) from corresponding control.

[2] TSH values to be used as control for PTU group.

TABLE 6. Free Thyroxine Index

ETU (ppm)	Sex	Days on Study 30	60	90
625	M	0.52	0.15	0.68
625	F	0.67	0.12	0.70
125	M	1.62	1.86	1.48
125	F	1.27	1.35	1.05
25	M	3.69	2.12	2.64
25	F	2.42	1.91	1.90
5	M	3.24	3.27	3.53
5	F	2.00	1.88	2.12
1	M	3.28	3.49	2.64
1	F	2.22	1.86	1.52
0	M	3.37	3.52	3.12
0	F	2.52	2.31	2.14
Amitrole (ppm)				
50	M	0.35	4.37	2.53
50	F	0.15	2.42	2.41
PTU (ppm)				
125	M	0.64	0.74	0.33
125	F	0.49	0.48	0.23

ETU

Rats ingesting ETU at 625 ppm showed a significant decrease in iodine uptake. The percent T-3 bound to TBG was lower than normal at the 30— and 90-day evaluations. Serum concentrations of T-3 and T-4 were also very substantially lower than the levels obtained for the control rats. The serum TSH concentration for the 625 ppm rats, measured only at the time of the 30-day evaluation, was increased to more than twice the serum TSH concentration observed for the control rats. Significant differences in free T-4 were also found as determined by the FTI.

The rate of iodine uptake, the percentage of T-3 bound to TBG, and the serum T-3 concentration in the rats receiving 125 ppm ETU were not significantly different from the control group. However, a statistically significant decrease in total serum T-4 concentration and in free serum T-4 measured at the 30-, 60- and 90-day evaluations was observed. The serum TSH concentration in the 125 ppm ETU rats at 30 days was approximately three times the concentration measured in the control rats.

With one exception, there were no significant measurable differences in iodine uptake, T-3 binding to TBG, or serum T-3, T-4, and TSH levels in the rats receiving ETU at either 25, 5 or 1 ppm for 30, 60, or 90 days. The rats ingesting 25 ppm ETU for 60 days showed a statistically significant increase in serum T-4 concentration. This was not evident at 30 or 90 days.

Amitrole

After 30 days of amitrole ingestion, very significantly decreased serum T-3 and T-4 levels were observed. The female rats from this group also had a decreased percent of T-3 bound to TBG. Both the male and female rats had an increased serum TSH concentration. While the decreased T-4 levels were still found after 30 days of amitrole ingestion, iodine uptake appeared to have returned to normal, probably as a result of biological compensation. At 60 and 90 days, normal values for all of the thyroid function measurements were noted.

Propylthiouracil

The rats which received PTU had decreased serum T-3 and T-4 concentrations at 30, 60, and 90 days. The serum TBG level was affected only in the 60- and 90-day test groups. Since the PTU study was added after the ETU study had started, TSH values were obtainable for the 60- and 90-day PTU test groups. Both groups had markedly elevated serum TSH levels.

At the conclusion of the study, gross necropsies were performed. There were no significant differences in organ weights in those rats fed 25, 5, 1, and 0 ppm ETU. However, thyroid hyperplasia was observed in all animals which received 625 and 125 ppm ETU, 125 ppm PTU, or 50 ppm amitrole in their diet for 30, 60, or 90 days.

The organ-weight-to-body-weight ratios were calculated. The spleen-to-body-weight ratio for the rats which ingested 625 ppm ETU was significantly decreased, while the ratios for the thyroid, brain, kidneys, testicles,

and pituitary were significantly higher than the corresponding control values. Increased thyroid-to-body-weight ratios were also found for the rats which received 125 ppm PTU, 50 ppm amitrole, or 125 ppm ETU.

Organ-weight-to-brain-weight ratios were also calculated. No significant differences were found between rats receiving 125, 25, 5, 1, and 0 ppm ETU. For those rats ingesting 625 ppm ETU, the ratio of heart, spleen, kidney, adrenals (female), and ovaries-to-brain-weight was significantly decreased, and the thyroid-to-brain-weight ratio was significantly increased compared to control values. The thyroid-to-brain-weight ratios in the rats which received 125 ppm ETU were also significantly increased in size at 30, 60, and 90 days.

An increased thyroid-to-brain-weight ratio was the only significant organ-to-brain-weight ratio change found in the 50 ppm amitrole test animals. The animals which received 125 ppm PTU for 30, 60, or 90 days showed a very significant decrease in organ-to-brain-weight ratios for heart, spleen, kidney, ovary, liver, and adrenals. These animals had a very significant increase in thyroid-to-brain-weight ratios compared to the control values.

Gross examinations revealed that the majority of the rats which received ETU at 125 or 625 ppm, as well as those animals which received PTU or amitrole for 30, 60, or 90 days, had enlarged red thyroids. Grossly visible patterns of centrilobular congestion were present in a few random rats fed amitrole or ETU. A summary of gross changes is presented in Table 7.

Microscopic examination of tissues was performed on thyroid and liver. The thyroids were graded for degree of hyperplasia using a slight modification of the procedure of Astwood (Astwood, 1943), as defined in Table 8. Varying degrees of thyroid microfollicular hyperplasia resulted at feeding levels of ETU above.25 ppm (Table 9). Relatively mild changes were noted in animals fed 125 ppm ETU for 30 days, compared to the moderate changes observed in the rats fed 625 ppm ETU for the same time period. Marked thyroid microfollicular hyperplasia and reduced colloid formation were found in animals fed amitrole or PTU for 30 days. At 60 days, the rats receiving the three highest levels of ETU (625, 125, and 50 ppm) and the amitrole treatment group showed moderate thyroid hyperplasia as compared to the 60-day PTU group, which was more marked in its thyroid hyperplastic response and reduced colloid production. Ninety days of dietary exposure to 625 ppm ETU also resulted in microfollicular hyperplasia, but not as severe as seen with PTU in the same time period. Numerous changes ranging from adenomatous hyperplasia of follicles to what appear to be true solid adenomas were present in the thryoids in the 625 ppm ETU group. No adenomas were observed in the thyroid sections taken from the other dietary groups.

No liver tumors were observed in the rats at any dosage level of ETU or in those animals which received PTU or amitrole. The majority of the livers examined microscopically had mild centrilobular changes described as follows: Portal triad areas had a smooth sheet of hepatocytes cut relatively across the nucleus and cytoplasm at about the same level giving well defined cell boundaries and cytoplasmic proportions. As the examination

TABLE 7. Abnormal Changes Observed For Liver, Skin and Thyroid[1]

Tissue Change	Days on Study	Sex	PTU 125	Amitrole 50	ETU 625	ETU 125	ETU 25	ETU 5	ETU 1	Control[2] 0
Enlarged Thyroids, Red	30	M	10	10	10	8	0	0	0	0
	30	F	10	10	5	2	0	0	0	0
Thyroids Normal Size but Red	30	M	0	0	0	0	3	0	0	0
	30	F	0	0	0	0	2	0	0	0
Centrilobular Congestion, liver	30	M	0	0	0	0	0	0	0	0
	30	F	0	0	0	0	0	0	0	0
Alopecia	30	M	0	0	8	0	0	0	0	0
	30	F	0	0	4	0	0	0	0	0
Small Adrenals	30	M	0	8	0	0	0	0	0	0
	30	F	0	2	0	0	0	0	0	0
Enlarged Thyroids, Red	60	M	10	5	5[3]	6	1	2	0	0
	60	F	10	2	9	1	0	2	0	0
Centrilobular Congestion, liver	60	M	0	2	0	2	3	2	0	1
	60	F	0	0	0	0	0	0	0	0
Alopecia	60	M	0	0	5	0	0	0	0	0
	60	F	0	0	9	0	0	0	0	0
Enlarged Thyroids, Red	90	M	10	10	8[4]	10	0	0	0	0
	90	F	10	5	6	6	0	0	0	0
Alopecia	90	M	0	0	6	0	0	0	0	0
	90	F	0	0	4	0	0	0	0	0
Centrilobular Congestion, liver	90	M	0	3	0	9	1	3	6	1
	90	F	0	2	0	0	1	0	2	0
Small Adrenals	90	M	8	0	0	0	0	0	0	0
	90	F	9	0	0	0	0	0	0	0
Adenocarcinoma of Salivary Gland	90	M	0	0	0	0	0	0	0	0
	90	F	1	0	0	0	0	0	0	0

[1] Presented as the number of animals with the indicated tissue change, out of the 10 animals evaluated.

[2] Twelve male and eleven female control rats were evaluated from 30 day group. Twelve male and twelve female rats were evaluated from the 60 and 90 day control groups.

[3] Five male rats and one female rat from the 625 ppm 60 day group died prior to the end of the study. Therefore, the values for this group are based on the evaluation of five male and one female rat.

[4] Two female rats from the 625 ppm group died prior to the end of the 90 days. Therefore, the values for females on this group are based on eight rats.

TABLE 8. Grading Of Degree Of Hyperplasia From Microscopic Appearance Of The Thyroid Gland

Grade of Hyperplasia	Description of Change in Morphology
0	Completely normal appearing gland.
>1	Very slightly altered lining cells and scalloping of colloid.
1	Distinct hyperplasia or hypertrophy of lining cells and a moderate reduction of colloid.
>2	Hyperplastic change of lining cells and folding of follicular wall with a moderate reduction of colloid.
2	Moderate hyperplasia of lining cells, increase in apparent number of micro-follicular follicles reducing apparent colloid to less than fifty percent of normal.
>3	More extensive microfollicular pattern but still retaining approximately twenty-five percent colloid.
3	Marked hyperplasia of lining cells with virtually all microfollicular follicles and nearly complete absence of colloid.

TABLE 9. Thyroid Hyperplasia Observed In ETU Test Animals

Degree of Hyperplasia	Days on Study	PTU	Amitrole	ETU					
		125	50	625	125	25	5	1	0
0	30	0	0	0	0	0	1	0	4
>1	30	0	0	0	0	9	6	7	9
1	30	0	0	2	18	11	13	13	10
>2	30	0	0	4	1	0	0	0	0
2	30	8	0	12	1	0	0	0	0
>3	30	3	5	2	0	0	0	0	0
3	30	9	15	0	0	0	0	0	0
0	60	0	0	0	1	7	4	1	5
>1	60	0	4	0	3	2	7	9	10
1	60	0	5	2	7	0	9	10	9
>2	60	0	8	2	5	7	0	0	0
2	60	0	3	10	2	4	0	0	0
>3	60	17	0	0	0	0	0	0	0
3	60	3	0	0	2	0	0	0	0
0	90	0	0	0	1	3	6	6	12
>1	90	0	5	0	0	8	8	6	9
1	90	0	14	1	14	9	6	8	3
>2	90	0	0	1	3	0	0	0	0
2	90	1	1	12	1	0	0	0	0
>3	90	10	0	3	0	0	0	0	0
3	90	9	0	1	1	0	0	0	0

approached the mid-zonal area progressing into the centrilobular area or small central veins, the pattern changed to less distinct cell borders of swollen cytoplasmic material with fewer apparent nuclei. The chord pattern was less distinct, and there was a mild increase in small polyploid nuclei. The general appearance was that of increased cytoplasm, fewer nuclei, and less orderly hepatic chords. The liver changes, although not accompanied by any clinical or biochemical disorder, were present in enough detail and magnitude to suggest the possibility of future hepatic involvement.

The data presented in this report show no significant measurable adverse effects of dietary ETU at dosages of 5 ppm and below. Significant toxic responses, observed as altered thyroid function and changes in thyroid morphology, resulted from the ingestion of ETU at 125 ppm or 625 ppm for 30, 60 or 90 days. The observable effects at 25 ppm were noted only at the 60-day interval and were not seen at 30 or 90 days. Based on these data, a dietary no-effect level in rats might be expected to be 25 ppm over the 90-day study, equivalent to an average daily intake ranging from 2.23 mg/kg (body weight) at week 1 to 1.78 mg/kg (body weight) at week 12.

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